Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization of selenomethionyl exo-β-1,3-galactanase from the basidiomycete *Phanerochaete chrysosporium*

Takuya Ishida,[a] Zui Fujimoto,[b] Hitomi Ichinose,[c] Kiyohiko Igarashi,[a] Satoshi Kaneko[c]* and Masahiro Samejima[a]

[a]Department of Biomaterials Sciences, Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, Japan, [b]Protein Research Unit, National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan, and [c]Food Biotechnology Division, National Food Research Institute, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan

Correspondence e-mail: sakaneko@affrc.go.jp

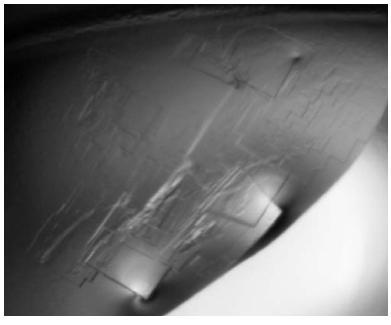
Received 4 September 2009
Accepted 21 October 2009

Exo-β-1,3-galactanase from *Phanerochaete chrysosporium* (Pc1,3Gal43A) consists of a glycoside hydrolase family 43 catalytic domain and a substrate-binding domain that belongs to carbohydrate-binding module family 35. It catalyzes the hydrolysis of β-1,3-galactan, which is the backbone of the arabinogalactan proteins; the C-terminal carbohydrate-binding module family 35 domain increases the local concentration of the enzyme around β-1,3-galactan by its high affinity for the substrate. To enable phase determination using the multi-wavelength anomalous dispersion method, selenomethionyl Pc1,3Gal43A was crystallized at 298 K using the hanging-drop vapour-diffusion method. The presence of selenium in the crystals was confirmed from the X-ray absorption spectrum. The crystals belonged to space group $P2_1$ and diffracted to 1.8 Å resolution.

1. Introduction

Arabinogalactan proteins (AGPs) are a family of complex proteoglycans that are widely distributed in higher plants and play important roles in plant growth and development such as root formation, promotion of somatic embryogenesis and attachment of pollen tubes (Seifert & Roberts, 2007). The AGPs are composed of a hydroxyproline-rich core protein and large amounts of carbohydrates. The carbohydrate moieties consist of a β-1,3-galactan backbone and β-1,6-galactan side chains, to which L-arabinose residues and other auxiliary sugars are attached, mainly through the O-3 atoms of galactose residues (the carbohydrates in this report belong to the D series unless otherwise indicated). Because of the large number of putative protein cores and the complex structure of the carbohydrate moieties, structural and functional studies of individual AGPs are rather challenging (Fincher *et al.*, 1974; Gaspar *et al.*, 2001). Hence, the structural simplification of AGPs by removal of the associated O-linked carbohydrates can provide a useful tool/methodology for investigation of AGPs.

Our laboratory has recently succeeded in cloning the exo-β-1,3-galactanase (EC 3.2.1.145) gene from the basidiomycete *Phanerochaete chrysosporium* (Pc1,3Gal43A; GenBank accession No. BAD98241; Ichinose *et al.*, 2005). The classification of the deduced amino-acid sequence of this enzyme in the CAZy database (http://www.cazy.org/; Cantarel *et al.*, 2009) revealed that it consists of a glycoside hydrolase (GH) family 43 catalytic domain and a substrate-binding domain belonging to carbohydrate-binding module (CBM) family 35 (formerly considered to be a member of CBM family 6; Ichinose *et al.*, 2005). Analysis of the recombinant protein expressed in the methylotrophic yeast *Pichia pastoris* demonstrated that Pc1,3Gal43A has a novel type of activity for GH family 43 and the sugar-binding properties of CBM family 35 (Ichinose *et al.*, 2005). Pc1,3Gal43A hydrolyzes the glycosidic linkages of β-1,3-linked galactose residues at the nonreducing end of β-1,3-galactan and



β-1,3-galactooligosaccharides and the C-terminal CBM family 35 domain is the first identified binding module to have an affinity for β-1,3-linked galactan (Ichinose *et al.*, 2005).

In addition to exo-β-1,3-galactanases, GH family 43 contains various GHs such as endo-α-L-arabinanases, exo-α-L-arabinanases, β-xylosidases, β-xylanases and exo-1,5-α-L-arabinofuranosidases. To date, structural analysis of GH family 43 enzymes has revealed that these enzymes have a common five-bladed β-propeller fold (Nurizzo *et al.*, 2002); however, no structures of exo-β-1,3-galactanases have been reported.

In the present study, we prepared crystals of selenomethionyl Pc1,3Gal43A for phase determination using the multiwavelength anomalous dispersion (MAD) method, as molecular replacement using native data for Pc1,3Gal43A had been unsuccessful.

2. Materials and methods

2.1. Expression and purification

In order to obtain selenomethionyl Pc1,3Gal43A, the *P. pastoris* transformant (Ichinose *et al.*, 2005) was cultivated in buffered minimal medium containing 1%(*w*/*v*) methanol, 0.1 mg ml^{-1} L-selenomethionine (SeMet), 0.09 mg ml^{-1} L-isoleucine, 0.09 mg ml^{-1} L-lysine and 0.6 mg ml^{-1} L-threonine as reported previously (Ishida *et al.*, 2009). The culture supernatant containing secreted protein was concentrated by ultrafiltration using a PM-10 membrane (Millipore, Billerica, Massachusetts, USA) and applied onto a Phenyl-Toyopearl 650S (Tosoh Corp., Tokyo, Japan) column (16 × 180 mm) equilibrated with 20 m*M* sodium acetate buffer pH 4.0. The protein was eluted with a linear gradient to 20 m*M* sodium acetate buffer pH 4.0 containing 1 *M* ammonium sulfate. The eluted recombinant protein was identified by SDS–PAGE and the relevant fractions were dialyzed against 20 m*M* sodium acetate buffer pH 4.0. The protein solution was applied onto an SP-Toyopearl 650S (Tosoh Corp., Tokyo, Japan) column (16 × 95 mm) equilibrated with 20 m*M* sodium acetate buffer pH 4.0 and the protein was eluted with a linear gradient to the same buffer containing 500 m*M* sodium chloride. The fractions containing recombinant protein were dialyzed against deionized water and concentrated to 11 mg ml^{-1} by ultrafiltration using Vivaspin 20 (Sartorius AG, Göttingen, Germany).

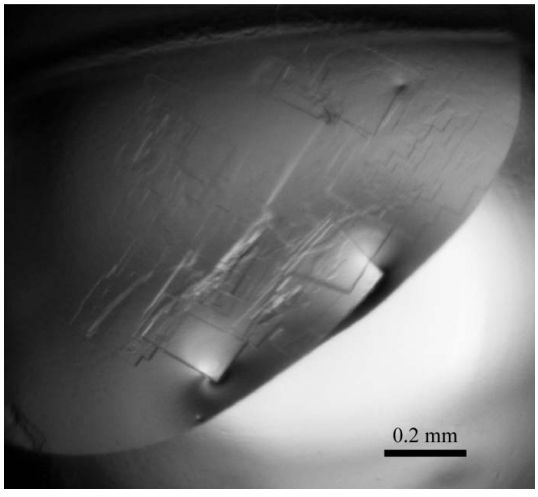


Figure 1
Crystals of SeMet-substituted Pc1,3Gal43A.

2.2. Crystallization

Sparse-matrix crystallization screening was performed with the Nextal JCSG+ suite (Qiagen, Hilden, Germany) using both the hanging-drop and sitting-drop vapour-diffusion methods with 50 and 100 µl reservoir solution, respectively. Each drop consisted of 1.0 µl (hanging-drop method) or 0.6 µl (sitting-drop method) protein solution and the same amount of reservoir solution and the crystallization plates were incubated at 298 K. Crystals were observed within 3–10 d under various conditions. The crystals used for data collection were obtained from a condition with reservoir composition 16%(*w*/*v*) polyethylene glycol (PEG) 10 000, 95 m*M* ammonium sulfate, 95 m*M* bis-tris pH 5.5 and 4.8%(*v*/*v*) glycerol.

2.3. Data collection and processing

An X-ray absorption spectrum and diffraction data for Se-MAD phasing were collected on beamline BL-6A of the Photon Factory (PF), High Energy Accelerator Research Organization, Tsukuba, Japan. The crystals were transferred to reservoir solution containing 20%(*v*/*v*) glycerol as a cryoprotectant and flash-cooled in a nitrogen stream at 95 K in nylon loops (Hampton Research, Aliso Viejo, California, USA). The X-ray absorption spectrum was determined by measuring the fluorescence signal perpendicular to the beam. A crystal from the same condition was used for multiple-wavelength data collection at 1.8 Å resolution. Diffraction data were collected with 10 s exposures using a Quantum 4R CCD detector (ADSC, California, USA) at peak, edge, high-remote and low-remote wavelengths of 0.97882, 0.97950, 0.96400 and 0.98300 Å, respectively. The oscillation angle was 1° over a total of 720° for the peak wavelength and 360° for the other wavelengths. The data were integrated and scaled using *DENZO* and *SCALEPACK* in the *HKL*-2000 package (Otwinowski & Minor, 1997).

3. Results and discussion

Selenomethionine was found to be successfully substituted into Pc1,3Gal43A using the *P. pastoris* expression system, although the amount of expressed protein was less than that of the native enzyme,

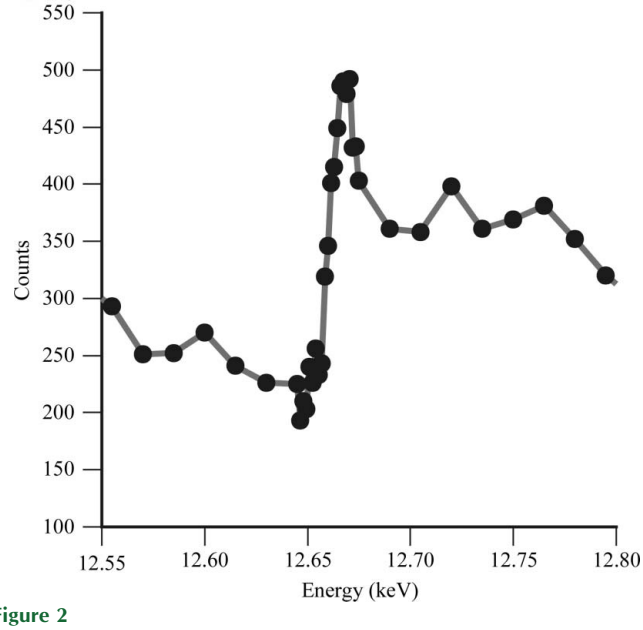


Figure 2
Se *K*-edge fluorescence scan of the crystal performed on beamline BL-6A at PF.

Table 1
Data-collection statistics for the SeMet Pc1,3Gal43A crystal.

Values in parentheses are for the highest resolution shell.

	Peak	Edge	High remote	Low remote
Space group	$P2_1$			
Unit-cell parameters (Å, °)	a = 66.4, b = 50.5, c = 75.8, $\alpha = \gamma$ = 90, β = 111.9			
Wavelength (Å)	0.97882	0.97950	0.96400	0.98300
Resolution range (Å)	50–1.80 (1.86–1.80)	50–2.00 (2.07–2.00)	50–2.00 (2.07–2.00)	50–2.00 (2.07–2.00)
$R_{\rm merge}$†	0.079 (0.672)	0.061 (0.307)	0.062 (0.307)	0.060 (0.303)
Completeness (%)	100.0 (99.9)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)
Multiplicity	14.0 (12.6)	7.2 (6.9)	7.2 (7.0)	7.2 (6.9)
Average $I/\sigma(I)$	36.6 (4.7)	30.9 (8.2)	31.3 (8.2)	30.8 (8.2)
Unique reflections	43643 (4353)	31744 (3139)	31780 (3146)	31760 (3144)
Observed reflections	613162	227158	228595	228381

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the weighted average intensity for all observations i of reflection hkl.

which was likely to be a consequence of the toxicity of selenomethionine and the stress resulting from cultivation in the minimal medium (Larsson *et al.*, 2002).

Despite the multidomain structure of Pc1,3Gal43A, crystals belonging to different space groups were obtained under various conditions in the first screening, suggesting that intact Pc1,3Gal43A has lower flexibility between the catalytic core and CBM family 35 domain than expected. This finding is consistent with recent articles on the crystallization and structural determination of other GH family 43 enzymes containing CBMs (Brüx *et al.*, 2006; Vandermarliere *et al.*, 2007, 2009; Fujimoto *et al.*, 2009).

Plate-like crystals (Fig. 1) of various sizes were obtained using both the hanging-drop and sitting-drop methods. The peak of 12.66 keV in the X-ray absorption spectrum corresponds to the Se *K* edge, indicating the presence of selenium in the crystal (Fig. 2). The crystal diffracted to a maximum resolution of 1.8 Å and belonged to space group $P2_1$. The processing statistics of the collected data are summarized in Table 1. The Matthews coefficient (Matthews, 1968) was calculated to be 2.47 Å^3 Da^{-1}, corresponding to a solvent content of 50.1% and suggesting the presence of one molecule in the asymmetric unit. Structure solution is in progress.

The use of synchrotron radiation for this work was approved by the Photon Factory Advisory Committee (proposal No. 2007G659); we thank the scientists and staff at the Photon Factory. This work was supported in part by a Grant-in-Aid for Scientific Research (to SK) from the Japan Society for the Promotion of Science and a Grant-in-Aid for Scientific Research 17380102 (to MS) from the Japanese Ministry of Education, Culture, Sports and Technology.

References

Brüx, C., Ben-David, A., Shallom-Shezifi, D., Leon, M., Niefind, K., Shoham, G., Shoham, Y. & Schomburg, D. (2006). *J. Mol. Biol.* **359**, 97–109.
Cantarel, B. L., Coutinho, P. M., Rancurel, C., Bernard, T., Lombard, V. & Henrissat, B. (2009). *Nucleic Acids Res.* **37**, D233–D238.
Fincher, G. B., Sawyer, W. H. & Stone, B. A. (1974). *Biochem. J.* **139**, 535–545.
Fujimoto, Z., Ichinose, H., Harazono, K., Honda, M., Uzura, A. & Kaneko, S. (2009). *Acta Cryst.* F**65**, 632–634.
Gaspar, Y., Johnson, K. L., McKenna, J. A., Bacic, A. & Schultz, C. J. (2001). *Plant Mol. Biol.* **47**, 161–176.
Ichinose, H., Yoshida, M., Kotake, T., Kuno, A., Igarashi, K., Tsumuraya, Y., Samejima, M., Hirabayashi, J., Kobayashi, H. & Kaneko, S. (2005). *J. Biol. Chem.* **280**, 25820–25829.
Ishida, T., Fushinobu, S., Kawai, R., Kitaoka, M., Igarashi, K. & Samejima, M. (2009). *J. Biol. Chem.* **284**, 10100–10109.
Larsson, A. M., Ståhlberg, J. & Jones, T. A. (2002). *Acta Cryst.* D**58**, 346–348.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Nurizzo, D., Turkenburg, J. P., Charnock, S. J., Roberts, S. M., Dodson, E. J., McKie, V. A., Taylor, E. J., Gilbert, H. J. & Davies, G. J. (2002). *Nature Struct. Biol.* **9**, 665–668.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Seifert, G. J. & Roberts, K. (2007). *Annu. Rev. Plant Biol.* **58**, 137–161.
Vandermarliere, E., Bourgois, T. M., Van Campenhout, S., Strelkov, S. V., Volckaert, G., Delcour, J. A., Courtin, C. M. & Rabijns, A. (2007). *Acta Cryst.* F**63**, 692–694.
Vandermarliere, E., Bourgois, T. M., Winn, M. D., van Campenhout, S., Volckaert, G., Delcour, J. A., Strelkov, S. V., Rabijns, A. & Courtin, C. M. (2009). *Biochem. J.* **418**, 39–47.